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Ultrastructural studies on microencapsulated oil droplets in aqueous gels and dried films of a new starch–oil composite[1]

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Combination of starch, water, and oil by a process utilizing an excess-steam jet cooker produces a new class of stable, oil-in-water dispersions. Examination of aqueous gels by light microscopy (LM) and transmission electron microscopy (TEM) and of dried thin films by scanning electron microscopy (SEM) shows that the oil is microencapsulated in the starch water matrix or in the dried starch matrix as droplets that are typically 1–10 μm in diameter. The size and distribution of oil droplets in cornstarch–soybean oil composites are determined by a number of factors, such as oil:starch ratio, the number of times the formulation is passed through the steam jet cooker during preparation, and the steam pressure used during cooking. Oil droplet size can be reduced by addition of protein or starch–oil composites from a previous cook to the formulation prior to cooking. Oil droplet distribution in these composites is observable not only in scanning electron micrographs of fracture surfaces, but also by light microscopy and by transmission electron microscopy of aqueous gels. Micrographs suggest the presence of a boundary layer surrounding the oil droplets which prevents them from coalescing. Published by Elsevier Science Limited.

INTRODUCTION

Jet cooking involves pumping an aqueous starch slurry through a narrow orifice where it is instantly solubilized by contact with high pressure steam (Klein & Brogly, 1981). Although jet cooking has been used commercially for decades to prepare starch solutions, the jet cooking of mixtures of starch with other monomeric and polymeric materials is a relatively new area of research that is currently being explored at our Research Center. The intense turbulence that results from condensation of high pressure steam and passage of excess steam through the cooker efficiently mixes starch and non-starch components and also results in shear-induced degradation of the polysaccharide. We have previously reported on a new type of stable dispersion produced by jet cooking a combination of starch, water, and various lipids (Eskins & Fanta, 1994; Fanta & Eskins, 1995; Knutson *et al.*, 1995). The resulting aqueous dispersions are stable and do not phase separate into oil and water components on prolonged standing. Freshly cooked dispersions, containing 10–20% solids, form soft gels upon standing that can be easily converted to pourable fluids by the application of heat and reformed into soft gels by cooling. The aqueous composites, which may contain up to 50% oil based on starch content, can be air dried into films or drum/spray dried into flakes or powders which are not oily to the touch. Dried compositions hydrate readily and are easily dispersed in water to form smooth, lump-free dispersions similar in nature to the original composites. The nature of these dispersions makes them suitable for a number of food and industrial applications, including uses in low-fat dairy and meat products, adhesives, foams, cosmetics, and drug delivery systems. The stability of these composites,

[1]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name of USDA implies no approval of the product to the exclusion of others that may also be suitable.

in the absence of emulsifiers, is unusual and contrary to past experience with jet-cooked starches (Trubiano, 1986) and different from mixtures of oils and starches (Seguchi, 1984) or oil and modified starches (Bangs & Reineccius, 1990; Inglett *et al.*, 1988). We have reported physical properties of the cooked composites and parameters of the cooking process in previous studies and now describe some ultrastructural characteristics of gels and thin films formed from the composite. This investigation sheds new light on the nature of the starch oil interaction and suggests structural features which might be responsible for the stability of the microencapsulated oil droplets.

Materials and methods

Composite preparation

In a typical preparation, 800 g of food grade corn starch was added to 3.0 L of water in a Waring blender. This suspension was well blended and 200 g of food grade soybean oil was added. In alternate methods of preparation, other lipids, resins, and proteins may be added. The blended mixture was continuously stirred and passed through a laboratory model excess-steam jet-cooker operating with either high pressure (120 psig) or low pressure (65 psig) inlet steam and sufficient back pressure to maintain 40 psig (140°C) in the hydroheater portion of the cooker. The hot material was then drum dried and passed through a Retsch mill to yield a fine powder. This material may be added to succeeding mixtures of starch, water, and oil ('add back procedure') to help stabilize these mixtures prior to jet-cooking. Aqueous gels were reconstituted from this powder by blending with hot water in a Waring blender at 20% solids. The hot fluid composites were either allowed to gel for examination by light microscopy (LM) or transmission electron microscopy (TEM) or poured into thin films and dried for examination by scanning electron microscopy (SEM).

Sample preparations for LM and TEM

Samples were poured into Petri dishes and held overnight at 4.0°C. Segments were cut out of the gel and sliced on a glass slide into 1-mm cubes. These were fixed in 2% glutaraldehyde in 0.05 M Na-phosphate buffer (pH 7.2) for 4 h at 25°C, rinsed 3 times in the buffer only, and post-fixed in 1% OsO_4 in the same buffer. Samples were dehydrated in an ethanol series, embedded in LR White acrylic resin, and polymerized at 60°C. Sections (1 μm thick) were cut with a glass knife on a Sorvall MT-2 ultramicrotome and adhered to gelatin-coated glass slides. Sections were stained with toluidine blue and photographed with a Zeiss bright-field microscope. For transmission electron microscopy, pale gold sections were stained with uranyl acetate and lead citrate and examined in a Hitachi H-500 electron microscope.

Sample preparation for SEM

Dried films of starch oil composite were fractured to expose the internal structure, then extracted in 100% ethanol for 24 h and further extracted with hexane for 1 h. Extracted samples were mounted fracture surface up and were sputter-coated with 200 Å gold palladium, then examined with a Jeol 6400 V scanning electron microscope.

Analysis of oil droplet size

Digital images of light micrographs or scanning electron micrographs were captured via a monochrome video camera onto a personal computer equipped with a video capture card. Oil droplets were quantified and their size was measured using Global Lab Image software (Data Translation, Marlboro, MA) after adjusting the images for optimum particle recognition. Analyses of light micrographs and SEM images were based on section fields of 40,800 μm^2 and fracture face fields of 21,000 μm^2, respectively. Values presented are the mean of 12 light micrographs or 3 SEM images per sample ± standard error.

RESULTS AND DISCUSSION

The size and distribution of oil droplets in these corn-starch–soybean oil composites are determined by a number of factors, perhaps the most important of which is the oil:starch ratio. Scanning electron micrographs in Fig. 1 show the effect of increasing the oil content from 5 to 40 parts per 100 parts of starch, by weight. Oil droplets, having been extracted from the fractured samples, appear as voids in the fracture surface. With 5 parts of oil per 100 parts starch (Fig. 1A), the diameter of oil droplets was approximately 2 μm or less. Oil droplets were larger when 20 parts of oil per 100 starch were used (Fig. 1B); and at a starch:oil ratio of 40:100 (Fig. 1C), most oil droplets were about 10 μm in diameter.

Light microscopy was also used to examine thin sections of aqueous composite gels that had been fixed by treatment with glutaraldehyde and osmium tetroxide. Figures 2A, B, and C show micrographs obtained from composites prepared from 10, 20 and 40 parts of soybean oil per 100 parts of starch, respectively. Although the size and distribution of oil droplets in these gels was somewhat different than that observed in air-dried films (Fig. 1), the droplets of oil were well dispersed and showed no evidence of agglomeration and separation.

A useful property of these starch–oil composites is their ability to provide stable oil–water dispersions when additional oil is added to an aqueous dispersion of the composite. For example, Fig. 2D shows a light

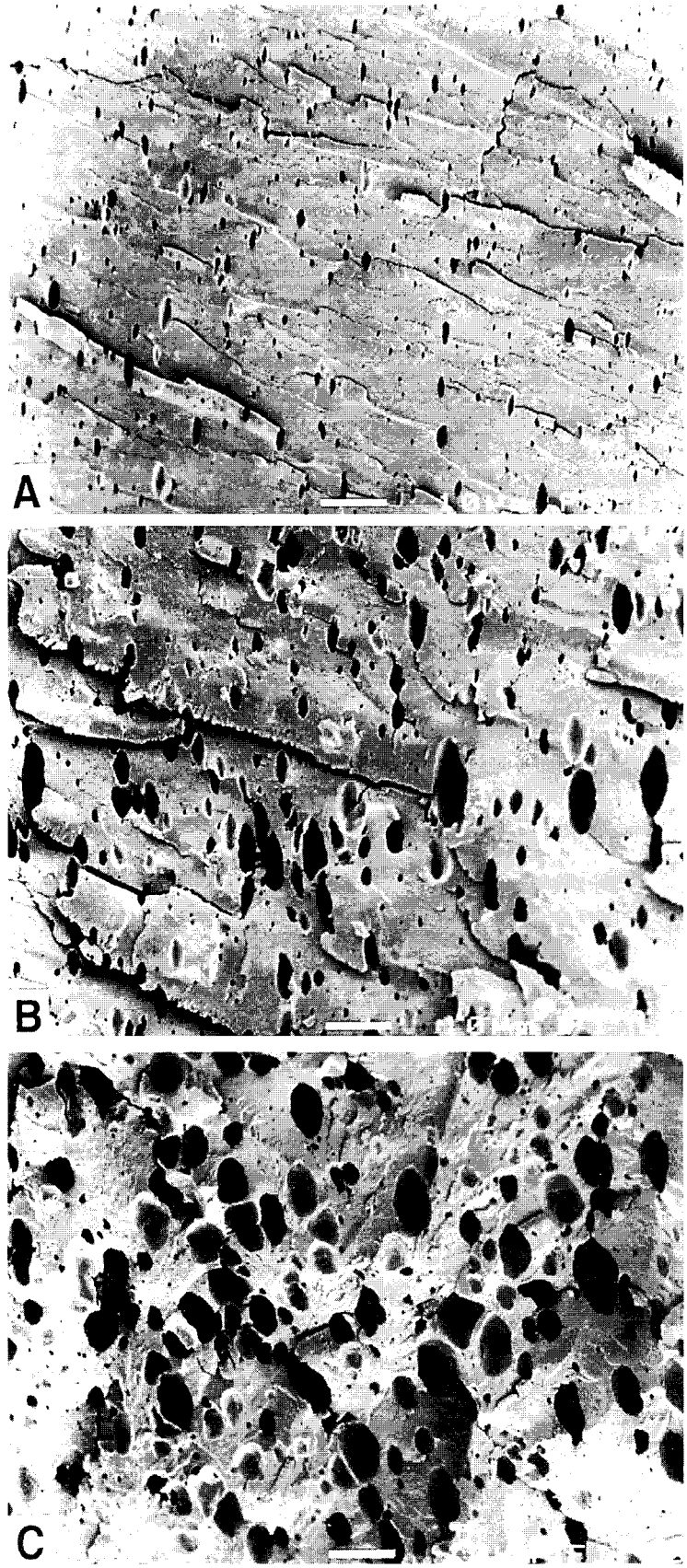

Fig. 1. SEM of fracture face of: (A) starch–soy oil composite (100 parts starch, 5 parts soy oil) dried to film and extracted with ethanol followed by hexane; (B) starch–soy oil composite (100 parts starch, 20 parts soy oil); (C) starch–soy oil composite (100 parts starch, 40 parts soy oil).

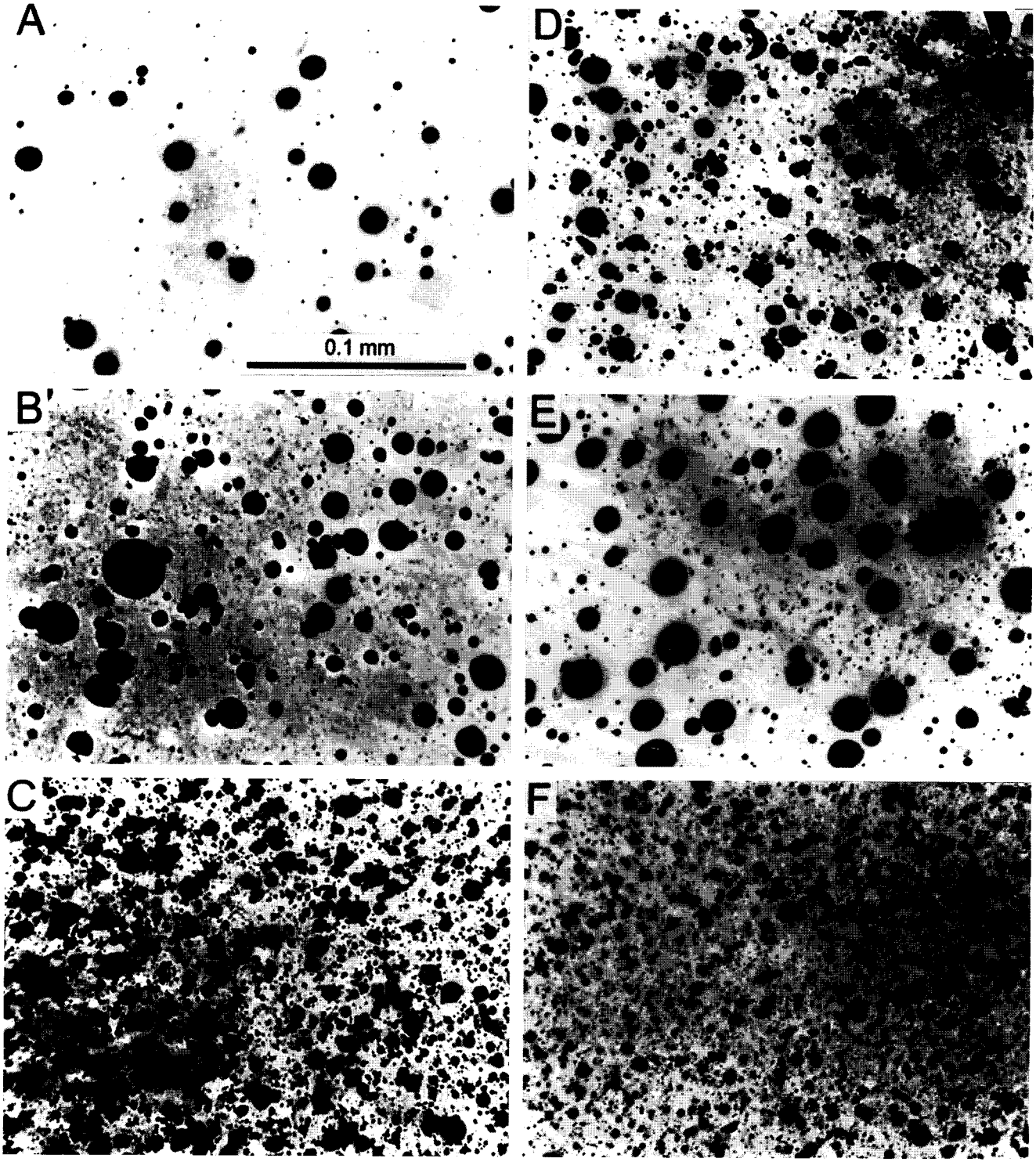

Fig. 2. Light micrographs of fixed, dehydrated, embedded, and sectioned samples of reconstituted starch–oil composite gels: (A) 10 parts oil, (B) 20 parts oil, (C) 40 parts oil per 100 parts starch by weight added before jet cooking. (D) 20 parts oil composite drum dried and extracted with hexane, then 10 parts oil added during reconstitution with water, (E) jet cooked starch to which 20 parts of oil added after cooking, (F) 40 parts oil composite drum dried, extracted with hexane, reconstituted with water.

micrograph of a gel prepared by: (1) drum drying a composite prepared from 20 parts of soybean oil per 100 parts of starch; (2) extracting the dried composite with hexane to remove loosely bound oil; (3) dispersing the extracted product in water; and (4) adding 10 parts of soybean oil back to the dispersion with rapid stirring in a Waring blender. Despite the additional oil added to the dispersion, oil droplets remained small and well dispersed within the starch gel matrix.

Figure 2E shows a light micrograph of a composite

gel prepared by: (1) jet cooking starch in the absence of oil; (2) blending soybean oil into the hot starch dispersion in a Waring blender in a ratio of 20 parts oil:100 parts starch; (3) drum drying the dispersion; and (4) reconstituting the drum-dried product in water. The similarity in the size and distribution of oil droplets in Figs 2B and E suggests that high intensity mixing of oil with jet-cooked starch solutions might also be used to prepare composites of this type. The co-jet cooking procedure, however, is simpler and would be more suitable for continuous processing.

Although some loosely-bound oil can be removed from drum-dried composites by hexane extraction, a major portion of the oil is resistant to extraction. For example, Fig. 2F is a light micrograph of a gel prepared from a drum-dried 40:100 soybean oil–starch composite after hexane extraction. The size and distribution of oil droplets is similar to that of a gel prepared from the same composite without prior extraction with hexane (Fig. 2C).

Four techniques were used to reduce the size of oil droplets formed within the aqueous starch matrix (Figs 3 and 4). These techniques are: (1) use of starch–oil composites to improve the initial dispersion of aqueous starch–oil mixtures prior to jet cooking; (2) addition of proteins such as wheat gluten or soybean protein isolates to the mixtures prior to cooking. Proteins are known to act as emulsifiers (Marsili, 1993) and would be expected to reduce droplet size; (3) use of high pressure steam (120 psig) vs low pressure steam (65 psig) to provide increased turbulence during jet cooking; (4) recooking or twice cooking of composites.

A scanning electron micrograph of a film fracture face prepared with 100 parts normal corn starch and 20 parts of soybean oil is shown in Fig. 3A. A standard field of this film's fracture face contained 328±32 holes which were occupied by oil droplets prior to extraction. Average area of individual oil droplets at the fracture face was 8.85±0.89 μm^2. A film prepared from jet-cooked composite containing 20 parts of soybean oil

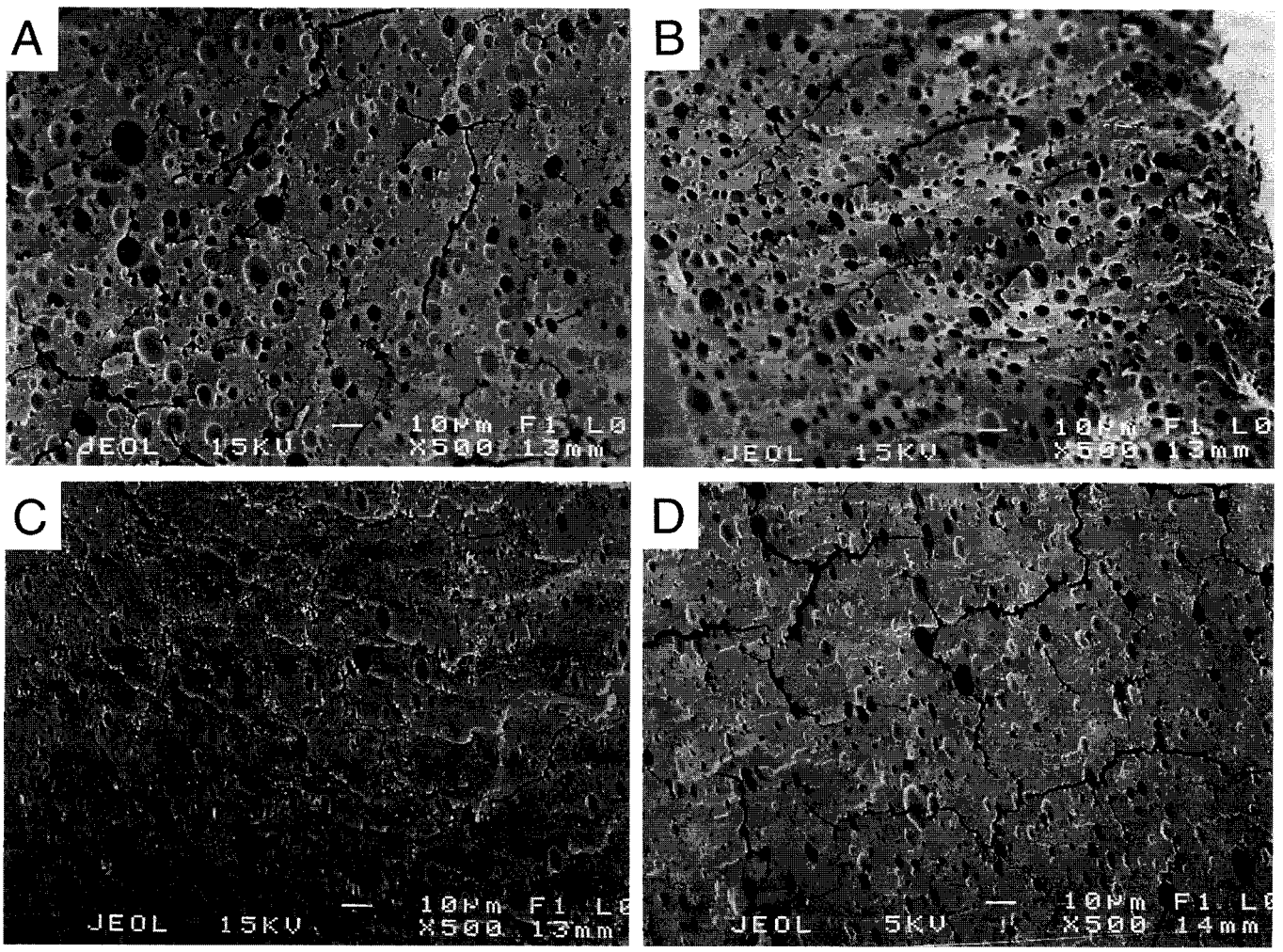

Fig. 3. SEM of fracture face of starch–soy oil composites (100:20) prepared by: (A) normal jet cooking process; (B) add back procedure using 20% by weight of dried starch–oil composite as helps disperse the oil prior to jet cooking; (C) addition of 20 parts wheat gluten protein to starch and oil prior to jet cooking; (D) jet cooking with high inlet steam pressure (120 psi inlet and 40 psi back pressure).

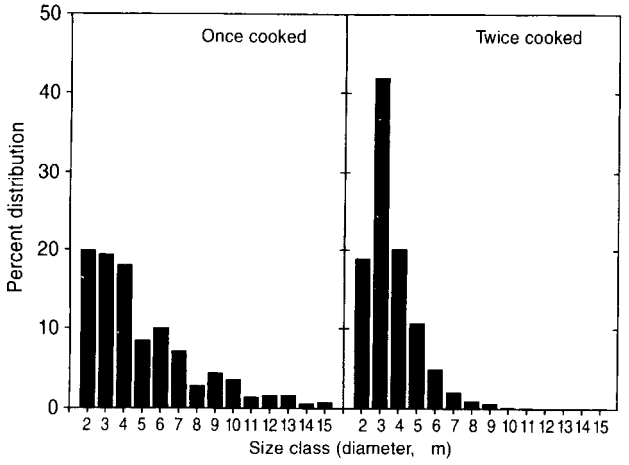

Fig. 4. Percent distribution of oil droplet size in single and twice cooked composites.

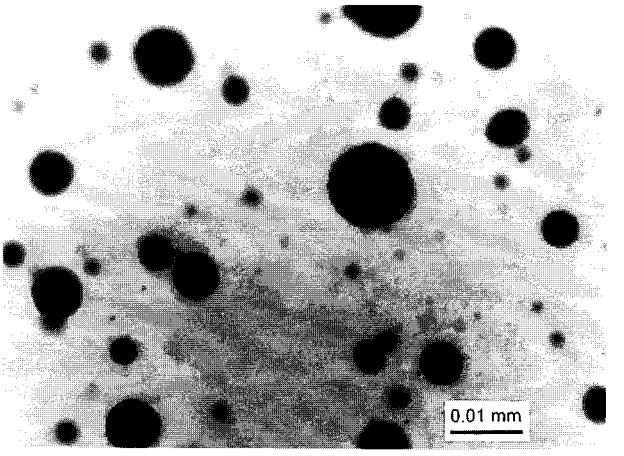

Fig. 5. Light micrograph of starch–oil gel (20% solids) reconstituted from drum dried solids added with mixing to water. Gels were stained with toluidene blue for starch and oil.

that had been prepared using the 'add-back' procedure is shown in Fig. 3B. The ability of these composites to act as dispersants for added oil not only inhibits the rapid separation of oil and water phases that normally occurs prior to cooking, but also reduces the size of oil droplets in the final product (compare Figs 3A and B). The amount of 'add-back' material incorporated in the formulation is typically 10–20% of the weight of starch used. This film fracture face had 421±26 holes per field and the average oil droplet area was 6.76±0.33 μm^2. A third film containing 100 parts starch, 20 parts of soybean oil and 20 parts of wheat gluten protein is shown in Fig. 3C. This film contained 714±21 holes per field with an average oil droplet area of 1.73±0.04 μm^2. The interaction of starch and protein (Dickinson, 1993) and protein and oil (Dickinson *et al.*, 1993) is well documented. Figure 3D shows a film fracture face of a composite containing 100 parts starch and 20 parts soybean oil prepared by jet cooking with high pressure steam (120 psi inlet pressure). Although the temperature and pressure in the hydroheater portion of the jet cooker is the same as for low pressure jet cooking, the increased turbulence and shearing in the cooker disperses the oil into more and smaller droplets. This film contained 552±26 holes per field with an average oil droplet area of 3.21±0.04 μm^2. Finally, a reduction in droplet size can also be achieved by simply passing the entire cooked dispersion more than once through the steam jet cooker. Figure 4 shows the distribution of droplet size in a twice-cooked composite, as compared to a composite prepared by a single pass through the cooker. The trend is to increased frequency of smaller droplets, especially in the 3 μm range.

Examination of aqueous gel slices at higher magnification with an oil emersion light microscope (Fig. 5) and with a transmission electron microscope (Fig. 6) shows that the various sized oil droplets appear to be surrounded by a boundary material, which is less dense than oil and stains differently than the surrounding starch. In many photographs, the boundary appears to be a bilayer composed of a lighter outer layer and a darker inner layer (Fig. 6) or a more darkly stained outer layer and a very lightly stained inner layer (Fig. 5). Although these apparent layers or boundaries may be artifacts of the fixing and staining process, we observed that added lipopolysaccharide with bound fluorescein indicator was concentrated in the boundary regions of the composite. In all photographs, the starch matrix is rigorously excluded and does not penetrate the boundary layers. Although adjacent droplets may be close to one another, there is no apparent coalescence or merging of boundary layers even after prolonged standing. This may explain the observed stability of dispersions of starch, oil, and water formed by our process. It also suggests exciting possibilities of using these microscopic droplets of oil as liposomes, microencapsulating agents, and drug-delivery systems, in which targeting information is imbedded in the boundary material surrounding the oil droplets.

A number of explanations can be proposed for the formation of these boundary layers, for example: — (1) Trace amounts of monoglyceride normally present in commercial triglyceride samples can form helical inclusion complexes with starch during steam jet cooking. These complexes may reside at the starch–lipid interface and act as emulsifiers for added oil. Traces of monoglycerides might also be produced by deesterification of triglycerides under the high temperature, high shear conditions of the jet cooking process. Lipid materials normally present as impurities in commercial starch samples could also yield starch–lipid complexes during jet cooking. (2) Trace amounts of protein present in commercial starch samples can act as emulsifiers for added oil. Corn starch, for example, contains roughly 0.3% protein (Watson, 1967). Zein, the most abundant

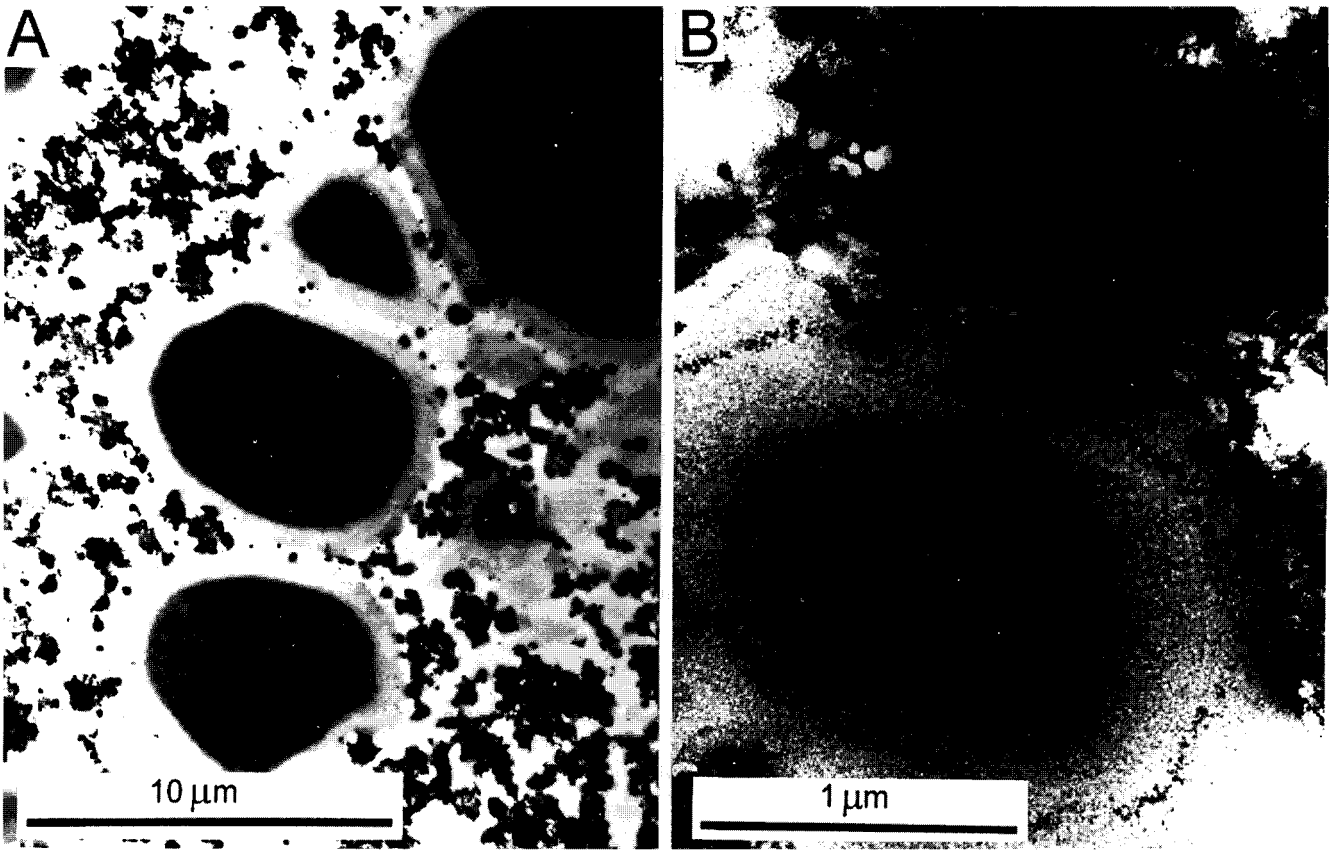

Fig. 6. Transmission electron micrograph (TEM) of starch–oil composite gel, showing (A) compression of adjacent oil droplets and lighter-staining border layer, and (B) darkly stained band within lightly stained outer layer and possible starch granule remnants in the aqueous phase region.

storage protein in corn endosperm, is not considered water-soluble, but it is possible that during the severe conditions of jet-cooking trace amounts of zein became solubilized. These hydrophobic proteins would probably cling to the surface of oil droplets and not remain soluble after cooling. (3) Complexes of starch with triglycerides might be produced in trace amounts under the high temperature and shear conditions of jet cooking, even though the sterically-hindered nature of the triglyceride molecule prevents their formation under ordinary conditions. The chemical nature of these boundary layers is currently under investigation.

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